REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

A. Claim Amendments.

Claim 18 is amended. Claims 20-23 and 32-35 are cancelled. Claims 25-31 were previously withdrawn. After amendment, Claims 18, 19 and 24 are pending.

The claims have been amended to be directed to treatment of heart failure in mammals using the S16E phospholamban (PLB) mutant. Limitations drawn to the mechanism of action are withdrawn as being inherent in the properties and corresponding activity of the molecule whose use is claimed. No new matter is added by this amendment, entry of which is therefore requested.

B. Response to Rejection under 35 USC Section 112, First Paragraph (enablement)

The pending claims remain rejected for lack of enablement. The asserted reason for the rejection is lack of proof that the claimed PLB mutants enhance SERCA2 activity in the heart in a way that would provide the claimed improvement in cardiac muscle contractility.

As to the Crystal, et al. reference relied upon in this regard by Applicants in the prior amendment, the Examiner contends that it acknowledged only that enhancing SERCA2 activity in the heart could have therapeutic value, but not that modulating phospholamban activity by use of the invention would achieve that goal (Action, at page 6). Applicants respectfully disagree, both with the characterization of the Crystal, et al. paper and with the assertion that use of the invention has not been shown to improve SERCA2 mediated cardiac contractility.

Firstly, the opinions expressed by Crystal, et al. specifically pertained to the invention *as it is claimed*. In particular, Crystal, et al. report the inventors' success in improving SERCA2 related cardiac contractility by *suppressing phospholamban activity* in a relevant animal model using *an expression construct encoding a PLB S16E mutant* as presently claimed. The authors opined that the work demonstrated promise for use in human gene therapy. Applicants therefore submit that Crystal, et al., established that those of skill in the art accept that a reasonable chance of success with no need for undue experimentation to enable use of the invention to improve cardiac contractility in the failing heart.

Specifically, Crystal, et al. stated:

> "...persistent activation of SERCA is an obvious therapeutic strategy for heart failure. But how can this be accomplished? In an attempt to meet this challenge, the [inventor] Chien laboratory used a gene therapy approach. They delivered a mutant form of phospholamban to the heart in a vector already shown to be effectie at transferring and persistently expressing genes in the heart:[ ] recombinant adeno-associated virus serotype 2 vector (AAV2). The mutant phospholamban (S16E) has a serine replaced by a glutamate at one of its two phosphorylation sites, mimicking phosphorylation of the serine...
>
> [t]heoretically, delivering an S16E form of phospholamban to heart cells should increase SERCA2 activity, and thus increase contractility. A variety of *in vitro* and acute *in vivo* models suggested that this strategy should work.[ ] However, heart failure is a chronic condition, and thus the critical question is whether this approach would persistently prevent heart failure? To address this question, Chien's group used an animal [mammalian] model of progressive cardiomyopathy and heart failure, the BIO14.6 hamster. [ ] With an adeno-associated virus vector, Chien and his co-workers were able to persistently express S16E phospholamban in the heart. They also showed that S16E phospholamban gene therapy, despite not addressing the primary cardiac abnormality in

> the BIO14.6 hamsters[1] [ ], [still] enhanced a variety of parameters associated with cardiac function…".

In other words, the study performed by the inventors established that S16E phospholamban enhanced SERCA2 activity and improved cardiac function, *irrespective* of whether other (non-SERCA2 related) abnormalities also existed in the treated heart. Thus, Crystal, et al. does not say, as the Office Action implies, that the effect of the mutant phospholamban of the invention on heart failure causatively related to abnormalities in the heart other than losses in SERCA2 activity. Rather, Crystal, et al. confirms the opposite: the invention is proven to work to improve SERCA2 related cardiac contractility *notwithstanding* the influence of other abnormalities.

Data generated in the hamster model of cardiomyopathy referred to by Crystal, et al. confirms the authors' impression of the invention's practicability. Following intra-coronary administration of an AAV-S16EPLB construct, the animals enjoyed "significantly enhanced cardiac contractility indicated by an approximately 33% increase in mean velocity of circumferential fiber shortening (mVcf) 6 days after transfection, while Ad-LacZ gave no significant effect. LVDd reduction also occurred in AdenoS16PLB transfected animals (6% decrease, $p<0.05$ vs. pre-operative measurement), whereas Ad-LacZ injected animals showed slight further enlargement in LV chamber, thereby documenting the short term efficacy of PLB inhibition." ('571 Application, Appendix A, at paragraph [0040]). Expression and a therapeutic response persisted for 3-6 months post-gene transfer, leading the inventors to conclude that "these data provide direct evidence that PLB inhibition can lead to chronic reversal of heart failure by employing a novel AAV mediated gene therapy strategy, even at stages of the disease

[1] The work in a hamster model of cardiomyopathy referenced by Crystal, et al. is described by the inventors in previously filed, co-pending application Serial No. 09/954,571 (US PG Pub. No. 2002/0032167, filed 9/11/2001). For ease of reference, the pertinent passages of the '571 Application are submitted herewith in Appendix A.

that correspond to severe near end-stage human heart failure (late NYHA Class III)." ('571 Patent, Appendix A, at paragraph [0041]).

The evidence of record therefore confirms what the Specification asserts: the S16E phospholamban molecule, delivered to the heart in an expression construct, enhances SERCA2 activity and improves cardiac contractility. Specifically, practice of the invention to treat heart failure by improving cardiac contractility has been demonstrated in an art-accepted model of human heart failure. Referring to the inventors' post-filing publication of the hamster model results, the Crystal, et al. paper concluded that these data are sufficient to demonstrate that the invention works as claimed to improve cardiac contractility.

Although, as Crystal, et al. suggested, some *further* experimentation may be desirable to optimize the invention for use in human gene therapy, no evidence of record indicates that the invention's usefulness for treating mammalian heart failure as claimed would require *undue* experimentation. Applicant respectfully submits that the foregoing establishes that the Specification enables use of the invention to suppress phospholamban activity in the heart, allowing SERCA2 mediated contractility to improve to treat heart failure, the target condition recited in all pending claims (see Claim 1).

Reconsideration and withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph is therefore respectfully requested.

CONCLUSION

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application. A change of correspondent's address for the undersigned is also submitted herewith.

No fee is believed due in connection with this Amendment. If any additional fees are due, the Commissioner is hereby authorized to charge any fees that may be required by this paper to Deposit Account No. 07-1896 referencing the above-identified attorney docket number. A copy of the Transmittal Sheet is attached.

Respectfully submitted,

Date: 9/10, 2007

Stacy L. Taylor
Registration No. 34,842
Telephone: (858) 677-1423
Facsimile: (858) 677-1465

DLA PIPER US LLP
4365 Executive Drive, Suite 1100
San Diego, California 92121-2133
USPTO Customer Number 28213

GT\6536480.1
328342-443

APPENDIX A

Excerpts from U.S. Patent Application No. 09/954,571

"[0040] Enhancement of cardiac contractility by administration of a dominant negative phospholamban. To directly compare the potential therapeutic effects of the recombinant adenovirus vector versus MV mediated expression of S16E, we generated AAV-S16EPLB and AAV-LacZ vectors (Xiao et al., 1998) and examined their respective effects on the progression of heart failure in BIO14.6 CM hamsters (5-34 weeks of age). At 5-6 weeks of age, the CM hamsters have clear evidence of cardiac dysfunction, with a significant decrease in fractional shortening (% FS) assessed by echocardiography (normal hamster 47.0.+-.6.7, n=15 vs. CM hamster 39.6.+-.7.2, n=14, P=0.006). Within the following 28 weeks, the CM hamsters developed rapidly progressive heart failure that is comparable to NYHA (New York Heart Association) class III, characterized by echocardiography with a marked decrease in % FS (at 18 weeks old, normal hamster 44.0.+-.4.9, n=14 vs. CM hamster 24.1.+-.4.3, n=10, P<0.001) and chamber dilation indicated by an increase in end-diastolic left ventricular chamber dimension (LVDd) (at 18 weeks old, normal hamster 4.46.+-.0.37, n=14 vs. CM hamster 5.28.+-.0.41, n=10, P<0.001). Intra-coronary administration of the AdenoS16EPLB significantly enhanced cardiac contractility indicated by an approximately 33% increase in mean velocity of circumferential fiber shortening (mVcf) 6 days after transfection, while Ad-LacZ gave no significant effect. LVDd reduction also occurred in AdenoS16PLB transfected animals (6% decrease, p<0.05 vs. pre-operative measurement), whereas Ad-LacZ injected animals showed slight further enlargement in LV chamber, thereby documenting the short term efficacy of PLB inhibition.

[0041] The long-term therapeutic efficacy of the intracoronary delivery of rAAV-S16EPLB in CM hamsters was also evaluated. Echocardiography demonstrated that the rAAV/S16EPLB gene transfer strongly suppressed the progressive impairment of cardiac contraction and chamber dilation found in the CM hamsters five weeks post-gene transfer... The maximum first derivatives of left ventricle (LV) pressure, LV max dP/dt was largely reversed toward the level of normal hamsters by rAAV/S16EPLB treatment at baseline as well as in response to the increased doses of the .beta.-adrenergic agonist, dobutamine... This effect of AAV-S16EPLB to mitigate the development of heart failure was further evident at 3-6 months post-gene transfer, with a substantial improvement in % FS (AAV-S16EPLB animals, 25.9.+-.5.7, n=13 vs. AAV-LacZ animals 20.2.+-.6.2, n=11, P<0.05)...and mVcf (AAV-S16EPLB animals, 3.4.+-.0.7, n=13 vs. AAV-lacZ animals 2.7.+-.0.7, n=11, P<0.05). The high fidelity left ventricular pressure measurement directly documented that the AAV mediated delivery of the pseudophosphorylation mutant PLB sustained its rescue effect on cardiac contractility for at 3 months post-gene delivery..., displaying an over 50% increase of LV max dP/dt in the S16EPLB-transferred

animals compared to LacZ controls. The persistent expression of the S16E peptide was evidenced by immuno-blotting analyses with an anti-PLB and anti-phospho 16-PLB antibodies (S16EPLB peptide vs. endogenous PLB=1.5-5:1, n=6), and southern blotting suggested that CMV-S16EPLB fusion gene is at least partially integrated in the host genome, which suggests that expression may indeed by long-term. Taken together, these data provide direct evidence that PLB inhibition can lead to chronic reversal of heart failure by employing a novel AAV mediated gene therapy strategy, even at stages of the disease that correspond to severe near end-stage human heart failure (late NYHA Class III).

[0042] Expression of a domainant negative phospholamban decreases damage in failing cardiac cells. Previous experimental and clinical studies have documented that chronic increases in contractility mediated by .beta.-adrenergic agonists or phosphodiesterase inhibitors can lead to the rapid progression of heart dysfunction in chronic heart failure. Furthermore, administration of .beta.-blockers may improve survival and the progression of clinical heart failure. The mechanisms which underlie this detrimental long term effect have been ascribed to catecholamine toxicity, and have raised the query as to whether the chronic stimulation of cardiac performance by catecholamine is inherently driving heart failure progression. By utilizing a novel intracoronary AAV system to deliver a pseudophosphorylated PLB mutant which constitutively activates cardiac contractility in the absence of cAMP stimulation, the current study challenges this notion. By short circuiting the .beta.-adrenergic system at a downstream point in the pathway that controls SR calcium cycling and cardiac contractility, we have shown that chronic increases in cardiac contractility can lead to a long-term reversal of cardiac dysfunction and a marked effect on heart failure progression. In this study, we provide evidence suggesting that one of the mechanisms for the sustained therapeutic effects of AAV-S16EPLB gene delivery in CM hamster model system relates to an effect on slowing the rate of myocyte cell death which underlies heart failure progression in this model. Histological analysis at 5 weeks post gene transfer revealed that cardiac interstitial fibrosis, which is progressive in the human cardiomyopathy was downregulated for an extended time period by AAV-S16EPLB gene delivery, and that the degree of cell injury, as assessed by the stability of the dystrophin complex, was significantly diminished in the animals treated with the rAAV/S16EPLB...".